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INVITED REVIEW

Male Endocrinology

Sex steroids and cardiovascular disease

Bu Beng Yeap

As men grow older, testosterone (T) levels decline and the significance of this change is debated. The evidence supporting a causal role for lower circulating T, or its metabolites dihydrotestosterone (DHT) and estradiol, in the genesis of atherosclerosis and cardiovascular disease (CVD) in men is limited. Observational studies associate low baseline T levels with carotid atherosclerosis, aortic and peripheral vascular disease, and with the incidence of cardiovascular events and mortality. Studies using mass spectrometry suggest that when total T is assayed optimally, calculation of free T might not necessarily improve risk stratification. There is limited evidence to support an association of estradiol with CVD. Interventional studies of T therapy in men with coronary artery disease have shown beneficial effects on exercise-induced myocardial ischemia. However, placebo-controlled, randomized clinical trials (RCTs) of T therapy in men with the prespecified outcomes of cardiovascular events or deaths are lacking. Meta-analyses of randomized controlled trials of T published up to 2010 found no increase in cardiovascular events, mortality, or prostate cancer with therapy. Recently, in a trial of older men with mobility limitations, men randomized to receive a substantial dose of T reported cardiovascular adverse effects. This phenomenon was not reported from a comparable trial where men received a more conservative dose of T, suggesting a prudent approach should be adopted when considering therapy in frail older men with existing CVD. Adequately powered RCTs of T in middle-aged and older men are needed to clarify whether or not hormonal intervention would reduce the incidence of CVD.
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INTRODUCTION

Several cross-sectional and longitudinal studies have documented lower testosterone (T) levels in older men, with the decline approximating 1% per annum and being more prominent in longitudinal studies.[1–6] Lower T levels in turn are associated with a range of poorer health outcomes in cross-sectional and longitudinal analyses.[7,8] However, recent studies have reported that in the absence of major illness, T levels in older men can be comparable to those found in younger men.[9,10] Therefore, lower T levels may be an age-associated phenomenon which contributes to the increasing burden of ill-health in older men, or may reflect preexisting disease thus functioning as a biomarker for poorer health during male ageing. The evidence for a causal role of lower circulating T, or its metabolites dihydrotestosterone (DHT) and estradiol (E2), in the genesis of cardiovascular disease (CVD) in men is incomplete. Observational studies associate low T levels with the presence of preclinical atherosclerosis and CVD.[8] Longitudinal studies have shown that low T levels at baseline predict increased mortality, including mortality from CVD.[11] However, placebo-controlled, randomized clinical trials (RCTs) of T therapy in men with the prespecified outcomes of CVD events or deaths are lacking.[12] This is understandable, as RCTs powered for CVD events would require large numbers of men to be treated and followed for extended periods of time. Therefore, while men with pathologically-based androgen deficiency should be considered for T therapy,[13] the role of T supplementation in older men with low-normal T levels in the absence of pituitary or testicular disease remains controversial. Additional studies are needed to clarify to extent to which T, DHT, and E2 are independent predictors of CVD-related outcomes, and to facilitate the design of future interventional studies of T in ageing men.

CIRCULATING TESTOSTERONE, FREE TESTOSTERONE, DIHYDROTESTOSTERONE AND ESTRADIOL

T circulates bound with high affinity to sex hormone binding globulin (SHBG) and with lower affinity to albumin, with a small fraction unbound or free.[14] Levels of SHBG are higher in older men, therefore levels of free T decline more steeply than total T as men's age increases.[1–6,15–17] There is ongoing debate over validity of the "free hormone hypothesis" and hence over the utility of either free T or non-SHBG-bound T ("bioavailable T") as markers of androgen status in older men.[18,19] As measurement of free T by equilibrium dialysis is technically demanding, it is commonly calculated from total T and SHBG.[20] However, calculations based on mass action equations may not reflect precisely free T measured using a reference method.[21,22] T is converted by the intracellular enzyme 5α-reductase into DHT, a more potent ligand for the androgen receptor, and by aromatase (*CYP19A1*) to E2, a ligand for the estrogen receptors α and β.[17] Both DHT and E2 can be regarded as sharing steroid hormone binding sites on SHBG with T.[21] Analogous to the trends seen for total and free T, free DHT and free E2 tend to decline more steeply with increasing age of men, while total DHT and total E2 levels are more stable.[1,4,5,15,23–25] Total T levels have often been measured using platform immunoassays, and these methods are vulnerable to loss of specificity and method-dependent bias.[26,27] Similar issues are seen with measurement of E2 by immunoassay, and would be expected for DHT.[28,29] While immunoassay can be preceded by solvent extraction and chromatography, mass spectrometry currently

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is the preferred assay methodology for sex steroids.[30] More recent studies have reported on distributions or associations of sex steroids in men using either gas chromatography-MS (GC-MS)[31,32] or liquid chromatography-tandem MS (LC-MS).[9,10,33–38] Consistent with previous studies based on immunoassays, in large population-based cohorts of middle-aged and older men, free T declines more steeply with age than total T in both cross-sectional[35] and longitudinal studies,[36] as does free E2 in comparison to total E2.[37] Age is independently associated with higher odds ratio (OR) for having total T, DHT, or E2 in the lowest quartile of values in older men.[38]

SEX STEROIDS AND CAROTID ATHEROSCLEROSIS

T may slow development of or progression of atherosclerosis by modulating effects on insulin resistance, inflammation, endothelial function, preclinical atherosclerosis or the vasculature.[39,40] Interestingly, Fogelberg *et al*. reported atherosclerosis occurring in rabbits treated with a non-aromatizable androgen, suggesting that T might be proatherogenic in the absence of conversion to E2.[41] Nathan *et al*. reported that effects of T to attenuate atherosclerosis in orchidectomized mice were abrogated by administration of an aromatase inhibitor.[42] These studies prompted consideration that E2 derived from aromatization of T might provide protection against atherosclerosis. By contrast, DHT acts via the androgen receptor to modulate angiogenesis in male, but not female endothelial cells.[43] Therefore, circulating E2 and T may exert distinct actions impacting on the development of atherosclerosis. Ultrasound measurement of carotid intima-media thickness (CIMT) provides a surrogate measure for the presence of atherosclerosis, being a predictor of future vascular events.[44] Observational studies in men examining the influence of sex steroids on either the presence or progression of CIMT are summarized in **Table 1**. Two cross-sectional studies in middle-aged and older men reported associations of lower total T with increased CIMT.[45,46] van den Beld *et al*. studied 403 men aged 73–94 years, reporting that lower total T was associated with CIMT.[45] Makinen *et al*. reported in 239 men aged 40–70 years that those men with symptoms consistent with androgen deficiency and either total T <9.8 nmol l^{-1} or elevated LH, had increased CIMT compared with controls.[46] Total T correlated inversely with CIMT. In another cross-sectional study of 1482 men aged 25–84 years, Svartberg *et al*. reported that lower total T as a continuous variable was associated with higher CIMT after adjustment for age and CVD risk factors but not BMI.[47] However, total T in the lowest quintile was associated with CIMT >1.04 mm in a fully-adjusted model. In these studies, E2 was not associated with CIMT.[45–47] Dorr *et al*. analyzed data from 1177 men aged (mean ± standard deviation) 62.2 ± 9.4 years and did not find an association of total T with CIMT; however, men with lower levels of T had a higher prevalence of carotid plaques.[48] Two recent cross-sectional studies provide supportive results. Soisson *et al*. noted that total and bioavailable T were inversely correlated with CIMT in 354 men with a mean age of 73.4 years.[49] There was an interaction with CRP, as in men with elevated CRP those with lower bioavailable T levels had increased CIMT. Tsujimura *et al*. found that free T was inversely associated with CIMT in 176 men aged 40–62 years.[50]

There have been relatively fewer longitudinal studies of sex steroids and progression of CIMT or carotid plaque. In one prospective study by Muller *et al*. of 195 older men who had CIMT measured in 1996 and again in 2000, lower free T was associated with progression of CIMT independently of CVD risk factors.[51] Of note, there was an association of higher total and free E2 with progression of CIMT, which was of borderline statistical significance. Tivesten *et al*. measured CIMT in 313 men at baseline (age 58 years) and after 3 years of follow-up, finding that both total and free E2 levels were associated with progression of CIMT.[52] Vikan *et al*. analyzed both CIMT and carotid plaque in the Tromso cohort, involving 2290 men in a cross-sectional and 1101 men in a longitudinal analysis.[53] Total T levels were inversely related to plaque area rather than CIMT, with no association of T with change in plaque area during follow-up. Therefore, there are observational data which associate low T levels with the presence of preclinical atherosclerosis as assessed by CIMT. However, longitudinal data are inconsistent, with individual studies implicating either low free T or higher total and free E2 with progression of CIMT. Overall, these cross-sectional and longitudinal studies support a relationship between low circulating T with CIMT and higher E2 with its progression; however, causality remains to be proven.

SEX STEROIDS AND AORTIC OR PERIPHERAL VASCULAR DISEASE

Observational studies of sex steroids in relation to aortic and peripheral vascular disease are shown in **Table 2**. Two studies have examined the association of sex steroids with disease of the abdominal aorta. In a study of 504 men aged ≥55 years, Hak *et al*. reported that low levels of total or bioavailable T were associated with aortic atherosclerosis manifested as calcified deposits detected by radiography.[54] In a

Table 1: Observational studies examining associations between sex hormones and preclinical atherosclerosis in middle-aged and older men

Study author and year [ref no.]	*Size (n of men)*	*Follow-up (year)*	*Age (year)*	*Results*
Van den Beld *et al*. 2003[45]	403	X	73-94	Total T inversely related to CIMT, E2 not associated
Makinen *et al*. 2005[46]	239	X	40-70	Androgen deficient men had higher CIMT, total T inversely correlated to CIMT, E2 not associated
Svartberg *et al*. 2006[47]	1482	X	25-84	Inverse association of total T with CIMT, not independent of BMI. E2 not associated
Dorr *et al*. 2009[48]	1177	X	62	Higher prevalence of carotid plaques in men with low total T levels. No relationship between T level and CIMT
Soisson *et al*. 2012[49]	354	X	≥65	Total and bioavailable T[a] inversely correlated with CMIT, E2 not associated. CIMT higher in men with low bioavailable T and CRP≥2 mg l^{-1}
Tsujimura *et al*. 2012[50]	176	X	≥40	Lower free T[b] associated with CIMT, total T not associated
Muller *et al*. 2004[51]	195	4	≥70	Free T[b] inversely related to progression of CIMT. Association of total and free E2 with CIMT progression of borderline significance
Tivesten *et al*. 2006[52]	313	3.2	58	Total and free E2 levels positively associated with progression of CIMT
Vikan *et al*. 2009[53]	1101	7	59	Inverse association between total T levels and total carotid plaque area. No longitudinal association of sex hormone levels and change in plaque area or CIMT

BMI: body mass index; CIMT: carotid intima-media thickness; E2: estradiol; T: testosterone; X: cross-sectional analysis; [a]Bioavailable T measured following ammonium sulfate precipitation of SHBG-bound T; [b]Free T measured using analog ligand radioimmunoassay; Unless otherwise specified total T and total E2 were measured by immunoassay; free T and free E2 were calculated; CRP: C-reactive protein.

subset of these men followed-up after 6.5 years those with total and bioavailable T in the middle and highest tertiles had less progression of calcific aortic plaque. A distinct manifestation of aortic vascular disease is abdominal aortic aneurysm (AAA). Men have a fivefold greater prevalence of AAA compared with women, and the presence of AAA is associated with mortality in older adults due to both aortic rupture and also other cardiovascular events.[61,62] Recently, increased abdominal aortic diameter, even below the threshold for definition of AAA, has been identified as a predictor of overall mortality and of incident cardiovascular events.[63,64] Yeap *et al.* studied the association of T with AAA in 3620 men aged 70–88 years.[55] In multivariate analysis adjusting for potential confounders, circulating free T was negatively associated with the presence of AAA, while luteinizing hormone (LH) was positively associated. Similarly, there was an inverse association of free T with aortic diameter, and a positive association of LH. Therefore, lower levels of T are biomarkers for aortic vascular disease, but the underlying mechanisms influencing this association need to be examined.

Vascular disease of the lower limbs typically presents with symptoms of intermittent claudication when occlusive atheromatous disease limits blood flow resulting in calf pain on exertion. Observational studies examining the association of sex steroids with the peripheral arterial disease (PAD) have utilized the presence of intermittent claudication with or without a reduction in the ankle: brachial index (ABI) to define the presence of PAD.[56–60] The ABI comprises the ratio of blood pressures measured over the posterior tibial artery at the ankle and the brachial artery in the arm, where an ABI <0.90 is used to define the presence of PAD.[57] Price *et al.* studied 40 men aged an average of 71.9 years, who had either intermittent claudication and an ABI ≤0.90 or asymptomatic PAD with ABI ≤0.85 in at least one leg compared with 41 controls.[56] There was no statistically significant difference in total or free T, E2, or SHBG between the two groups, although this could have been influenced by the small sample size. Tivesten *et al.* reported a cross-sectional analysis of 2784 men aged an average of 75.4 years, in which free T was positively associated with ABI, while free E2 was negatively associated.[57] Men with total or free T in the lowest quartile had increased adjusted ORs for PAD defined as ABI <0.90, as did men with free E2 in the highest quartile of values. Haring *et al.* studied 1422 men aged an average of 61.0 years using LC-MS to assay total T and E2.[58] Free T in the lowest quartile of values was associated with ABI <0.90. Total T or SHBG were associated with PAD defined as the composite of ABI <0.90, intermittent claudication, or lower extremity revascularization in age-adjusted but not in multivariable-adjusted models. Men with higher levels of SHBG at baseline were more likely to have a decline of at least 0.15 in ABI after 6.5 years of follow-up.[58] Maggio *et al.* reported a cross-sectional analysis of 419 men aged on average 74.2 years.[59] SHBG was negatively associated with PAD defined as ABI <0.90, but this association was not significant after adjusting for E2 and T.[59] Yeap *et al.* studied 2703 men aged 70–89 years in whom T, DHT, and E2 were measured using LC-MS.[60] Higher total T or DHT was associated with reduced risk of having intermittent claudication after comprehensive adjustment for cardiovascular risk factors, while E2 was not associated. In this analysis, calculation of free T did not change the risk stratification provided by total T alone. Higher SHBG was also associated with reduced OR of intermittent claudication; however, when total T and SHBG were included in the same model, the association with total T remained significant but the association with SHBG was attenuated.[60] Therefore, data from a large cohort study where sex steroids were measured using LC-MS support an association of higher total T or DHT with lower risk of clinically manifested PAD. The apparent association of SHBG with intermittent claudication reflects the correlation of total T with SHBG, while the contribution of E2 to risk of PAD remains unclear.

SEX STEROIDS AND CARDIOVASCULAR DISEASE EVENTS

Observational studies of sex hormones with the endpoint of CVD-related events are shown in **Table 3**. Earlier case-control studies with limited numbers of cases and controls had not shown differences in baseline T or E2 levels in men who subsequently experienced CVD events compared with controls.[65–67] The Caerphilly study of 2512 men aged 45–59 years followed-up for 16.5 years reported a trend across quintiles of cortisol: T ratio for incident ischemic heart disease.[68] This was statistically significant following adjustment for age, but not after adjustment for blood pressure, lipids, glucose, and insulin levels. There are inconsistent data for E2. Arnlov *et al.* analyzed 2084 middle-aged men without preexisting CVD from the Framingham Heart Study, followed-up for 10 years.[69] Men with total E2 in the highest quartile had lower incidence of fatal and nonfatal CVD events compared with men in the lowest quintile (hazard ratio (HR) =0.67, 95% confidence interval (CI) = 0.49–0.91). However, Abbott *et al.* in a study of 2197 older men followed-up till 7 years found that men with total E2 in the highest quintile had the greatest risk of stroke (HR = 2.2, 95% CI = 1.5–3.4).[70] Neither study found any significant association of T with their respective outcome measures. Vikan *et al.* found no association of total or free T, or total E2, with incident myocardial infarction in 1318 middle-aged men from the Tromso study followed-up for 9.1 years.[71] All three studies utilized immunoassays for measurement of T and E2.[69–71]

Table 2: Observational studies examining associations between sex hormones and aortic or peripheral vascular disease in middle-aged and older men

Study author and year [ref no.]	*Size (n of men)*	*Follow-up (year)*	*Age (year)*	*Results*
Hak *et al.* 2002[54]	504	6.5	≥55	Higher total and bioavailable T associated with reduced prevalence and less progression of abdominal aortic calcification
Yeap *et al.* 2010[55]	3620	X	70-88	Lower free T associated with abdominal aortic aneurysm and with aortic diameter as continuous variable
Price *et al.* 1997[56]	40 and 41	C/C	55-74	Total and free T[a], total E2 were not different in men with intermittent claudication and/or reduced ABI *vs* controls
Tivesten *et al.* 2007[57]	2784	X	69-80	Lower free T or higher free E2 associated with ABI<0.90
Haring *et al.* 2011[58]	1422	X, 6.7	61	Lower free T[b] associated with lower ABI. Lower SHBG associated with decline in ABI during follow-up
Maggio *et al.* 2012[59]	419	X	≥65	Low SHBG associated with PAD (attenuated on adjustment for total T and E2)
Yeap *et al.* 2013[60]	2703	X	70-89	Higher total T[c] or DHT associated with reduced risk of having intermittent claudication. E2 was not associated

ABI: ankle brachial index; C/C: case/Control study; DHT: dihydrotestosterone; E2: estradiol; PAD: peripheral arterial disease; SHBG: sex hormone binding globulin; T: testosterone; X: cross-sectional analysis; [a]Free T calculated as index of total T/SHBG; [b]T and E2 measured using liquid chromatography-tandem mass spectrometry (LC-MS); [c]T; DHT and E2 measured using LC-MS; Unless otherwise specified total T and total E2 were measured by immunoassay; free or bioavailable T; and free E2 were calculated

Table 3: Observational studies examining associations between sex steroid levels and cardiovascular events in middle-aged and older men

Study author and year [ref. no.]	*Size (n of men)*	*Follow-up (year)*	*Age (year)*	*Results*
Cauley *et al.* 1987[65]	163, 163	6-8	48	Nested case-control study. No differences in total or free T, or total or free E2 between men with major coronary event *vs* controls
Phillips *et al.* 1988[66]	96, 96	5-9	52-74	Nested case-control study. No differences in mean T or E2 between men who had MI *vs* controls
Hautanen *et al.* 1994[67]	62, 97	4	49.6, 47.0	Nested case-control study. No difference in T levels between men with cardiac events *vs* controls
Smith *et al.* 2005[68]	2512	16.5	45-59	482 deaths, 192 fatal, and 128 non-fatal IHD events. Higher cortisol: T ratio associated with IHD deaths and IHD events in age, but not multivariable adjusted analyses
Arnlov *et al.* 2006[69]	2084	10	56	386 had first cardiovascular event. Higher total E2 at baseline associated with lower incidence of CVD events. T not associated
Abbott *et al.* 2007[70]	2197	≤7	71-93	124 had first stroke. Baseline E2 in top quintile (≥125 pmol l^{-1}) associated with higher risk, total T not associated
Vikan *et al.* 2009[71]	1318	9.1	59.6	146 men had first ever MI. No association of total or free T or total E2 with incident MI
Yeap *et al.* 2009[72]	3443	3.5	≥70	First stroke or TIA occurred in 119 men. Total and free T in the lowest quartiles (<11.7 nmol l^{-1} and <222 pmol l^{-1}) predicted increased incidence of stroke or TIA (HR 1.99 and 1.69)
Akishita *et al.* 2009[73]	171	6.4	48	20 CVD events. Men in lowest tertile of total T (<14.2 nmol l^{-1}) had higher CVD event risk (HR 4.61)
Hyde *et al.* 2011[74]	3637	5.1	70-88	618 men experienced IHD event. Higher LH associated with incident IHD
Ohlsson *et al.* 2011[75]	2416	5	69-81	485 CVD events. Men with total T[a] in highest quartile (≥19 mol l^{-1}) had lower risk of CVD event (HR 0.77)
Haring *et al.* 2013[76]	254	5, 10	75.5	No associations of baseline total T or total E2 with incident CVD events

CVD: cardiovascular disease; E2: estradiol; HR: hazard ratio; IHD: ischemic heart disease; MI: myocardial infarction; T: testosterone; TIA: transient ischemic attack. [a]Total T measured using gas chromatography-mass spectrometry (GC-MS). Note: the studies by Vikan *et al.*;[71] and Haring *et al.*;[76] also reported mortality outcomes which are shown in **Table 4**. Results are from cohort studies unless otherwise specified. Unless otherwise specified total T and total E2 were measured by immunoassay; free or bioavailable T and free E2 were calculated

More recent studies have documented associations of lower T levels with incident CVD events.[72–75] Yeap *et al.* studied 3443 men aged ≥70 years followed-up for 3.5 years.[72] After adjustment for covariates including age, waist-hip ratio, waist circumference, smoking, hypertension, dyslipidemia, and medical comorbidity; men with total T in the lowest quartile of values (<11.7 nmol l^{-1}) experienced an increased incidence of stroke or transient ischemic attack (HR = 1.99, 95% CI = 1.33–2.99). Akishita *et al.* reported a smaller study of 171 middle-aged men with risk factors for coronary disease followed-up for 77 months.[73] Men with total T in the lowest tertile had a fourfold higher risk of CVD events in the fully adjusted analysis, although event numbers were small and the confidence intervals wide (1.02–21.04). Hyde *et al.* performed an analysis in older men using the endpoint of hospitalizations or deaths due to ischemic heart disease (IHD).[74] Men with higher baseline total or free T levels experienced fewer IHD events (HR = 0.89; 95% CI = 0.82–0.97 and HR = 0.86; 95% CI = 0.79–0.94 per one SD increase in total and free T, respectively). These associations were maintained after adjustment for age and waist: hip ratio, but did not persist after adjustment for prevalent IHD or other cardiovascular risk factors. Higher LH levels were associated with reduced event-free survival in both univariate (HR = 1.15; 95% CI = 1.08–1.22) and adjusted analyses (HR = 1.08; 95% CI = 1.01–1.15).[74] Ohlsson *et al.* used GC-MS to measure baseline total T in 2416 older men followed-up for 5.1 years.[75] Men with total T in the highest quartile of values (≥19.1 nmol l^{-1}) had a lower risk of CVD events (HR = 0.70, 95% CI = 0.56–0.88). An apparent association of higher SHBG with reduced incidence of CVD events was attenuated by inclusion of total T in the model, whereas the association of higher total T with reduced incidence of CVD events persisted after inclusion of SHBG.[75] Haring *et al.* studied 254 older men followed at 5- and 10-year intervals.[76] There was no association of baseline total T or total E2 with incident CVD events, nor were trajectories of these hormones associated with this outcome. Therefore, more recent and larger cohort studies in older men with greater numbers of outcome events have reported associations of lower total T with increased incidence of CVD events. There are fewer studies available in which associations of E2 have been studied, and the findings from these studies are inconsistent.

TESTOSTERONE AND MORTALITY

The association of sex steroids with mortality is a highly topical subject. Most of the studies to date have examined associations of endogenous circulating T with mortality, with a minority incorporating results for DHT or E2, as shown in **Table 4**. Shores *et al.* identified male veterans over the age of 40 years via a clinical database and reported that who had either a low total T (<8.7 nmol l^{-1}) or free T (n = 166) had increased mortality compared to those with equivocal (n = 240) or normal levels (n = 452) during 4.3 years follow-up.[77] A case-control analysis from the EPIC-Norfolk study found that higher total T levels were associated with lower all-cause and CVD-related mortality.[78] Araujo *et al.* reported a longitudinal analysis from the Massachusetts Male Aging Study involving 1686 men followed-up for 15.3 years.[79] In that study, lower free T was associated with reduced IHD mortality (lowest *vs* highest quintile, relative risk 0.45, 95% CI 0.23–0.89) and increased respiratory mortality albeit with wide confidence intervals (lowest *vs* highest quintile, relative risk 5.02, 95% CI 1.09–23.09). An association of lower DHT with higher IHD mortality was not statistically robust (lowest *vs* highest quintile, relative risk 1.88, 95% CI 0.94–3.75).[79] Maggio *et al.* studied 410 men aged ≥65 years followed-up for 6 years and reported higher mortality only in those men with low levels of multiple anabolic hormones.[80] However, subsequent cohort studies in mostly older men have supported the association of lower androgen levels with higher mortality.[31,71,82,84–87]

Lehtonen *et al.* reported a longitudinal study of 187 men aged 71–72 years, in which total T was inversely associated with 10-year mortality.[82] Lower total T was associated with higher mortality in older men with type 2 diabetes and stable coronary artery disease.[84] In the study by Vikan *et al.*[71] men with free T in the lowest quartile had higher all-cause mortality (HR 1.24, 95% CI 1.01–1.54). Tivesten *et al.* reported findings from the MrOS Sweden cohort of 3014 men aged 69–80 years followed-up for 4.5 years.[31] Total T and E2 measured using GC-MS were available for 2639 of these men. Total T or E2 in the lowest quartile of values were associated with higher mortality (HR 1.46 and 1.33 in model containing both hormones).[31] Men with lower total T and E2 had the highest all-cause mortality (HR 1.96). Interestingly, lower total T

Table 4: Observational studies examining associations between sex steroids and mortality in middle-aged and older men

Study author and year [ref. no.]	*Size (n of men)*	*Follow-up (year)*	*Age (year)*	*Results*
Shores *et al.* 2006[77]	858	4.3	≥40	208 deaths. Men with two or more low T levels (total T<8.7 nmol l^{-1} or free T<0.03 nmol l^{-1}) had higher mortality (HR 1.88)
Khaw *et al.* 2007[78]	825 and 1489	≤10	40-79	825 deaths, 1489 controls. Total T inversely related to mortality from all causes, CVD and cancer. A 6 nmol l^{-1} (1 SD) increase in total T was associated with mortality (OR 0.81)
Araujo *et al.* 2007[79]	1686	15.3	40-70	395 deaths. Higher free T associated with higher IHD mortality (relative risk 0.80 per 1 SD lower free T). Equivocal association of lower DHT with IHD mortality
Maggio *et al.* 2007[80]	410	6	≥65	126 deaths. Combination of bioavailable T, insulin-like growth factor-I and dehydroepiandrosterone sulfate in lowest quartiles associated with higher mortality
Laughlin *et al.* 2008[81]	794	11.8	50-91	538 deaths. Total T in the lowest quartile (<8.4 nmol l^{-1}) predicted increased mortality from all causes (HR 1.44) and from CVD and respiratory causes
Lehtonen *et al.* 2008[82]	187	10	71-72	68 deaths. T inversely associated with mortality
Vikan *et al.* 2009[71]	1568	≤13	59.6	395 deaths (130 from CVD and 80 from IHD). Free T in the lowest quartile (<158 pmol l^{-1}) predicted higher overall mortality (HR 1.24), total T not associated
Tivesten *et al.* 2009[31]	3014	4.5	75	383 deaths. Total T[a] and E2 levels in the lowest quartiles predicted mortality (HR 1.46 and 1.33, respectively). Risk of death nearly doubled (HR 1.96) in men with low levels of both total T and E2
Szulc *et al.* 2009[83]	782	10	≥50	Higher total E2 predicted increased mortality after the 3rd year (HR 1.21 per 1 SD increase, HR 1.80, 2.83 for Q3, Q4 *vs* Q1)
Ponikowska *et al.* 2010[84]	153	4	65	Men with type 2 diabetes and stable coronary artery disease. Low total T (≤10th percentile of healthy peers) predicted CVD mortality (HR 2.39)
Menke *et al.* 2010[85]	1114	18	≥20	103 deaths, 42 from CVD. Difference between 90th and 10th percentiles for free T associated with overall and CVD mortality in first 9 years of follow-up (HR 1.43 and 1.53, respectively). Difference for total E2 associated with CVD mortality (HR 2.40)
Haring *et al.* 2010[86]	1954	7.2	20-79	195 deaths. Total T<8.7 nmol l^{-1} associated with increased all-cause and CVD mortality (HR 1.9 and 2.6) and cancer death (HR 3.5)
Hyde *et al.* 2012[87]	3637	5.1	70-88	605 deaths, 207 from CVD. Lower free T (100 *vs* 280 pmol l^{-1}) predicted all-cause and CVD mortality (HR 1.6 and 1.7)
Haring *et al.* 2013[76]	254	5, 10	75.5	Higher baseline total T associated with lower 5 years, but not 10 years mortality risk. E2 not associated

CVD: cardiovascular disease; E2: estradiol; HR: hazard ratio; IHD: ischemic heart disease; MI: myocardial infarction; OR: odds ratio; Q: quartile; SD: standard deviation; T: testosterone. [a]T and E2 measured using GC-MS. Unless otherwise specified total T; DHT; and E2 were measured by immunoassay; free T; and free E2 were calculated

or E2 were associated with deaths from non-CVD causes, but not with deaths from CVD.[31] Szulc *et al.* followed 782 men aged 50 and older for 10 years.[83] Higher total E2 predicted increased mortality (HR 1.17 per 1 SD increase, 1.71 for highest quartile during entire follow-up period). Therefore, these studies indicated that lower total or free T levels were associated with mortality in older men, but with discordant results for cause-specific mortality and for associations of E2.

The most recent longitudinal cohort studies published since 2010 have addressed the relationship of T to all-cause and CVD-related mortality in younger, middle-aged, and older men[85,86] and older men specifically.[87] Menke *et al.* studied 1114 men from the Third National Health and Nutrition Examination Study aged ≥20 years followed for up to 18 years.[85] A decrease in free T equivalent to the difference between the 90th and 10th percentiles was associated with all-cause and CVD mortality in the first 9 years of follow-up (HR 1.43 and 1.53, respectively), as was an equivalent decrease in total E2 with CVD deaths within 9 years (HR 2.40). For deaths between 9 and 18 years of follow-up, men with total T in the second tertile had lower all-cause mortality (HR 0.35, 95% CI 0.15–0.81).[85] Haring *et al.* reported results from the Pomerania study of 1954 men aged 20–79 years followed-up for 7.2 years.[86] In that study, total T <8.7 nmol l^{-1} was associated with all-cause, CVD, and cancer mortality (HR 1.9, 2.5, and 3.5, respectively). The association with all-cause mortality was robust to the exclusion of deaths within the 1st year.[86] However, the association with cancer deaths raises the issue of whether low T levels in this cohort might have reflected existing ill-health. Hyde *et al.* reported results from the Health in Men study in which 3637 men aged 70–88 years were followed-up for 5.1 years.[87] Lower free T (100 *vs* 280 pmol l^{-1}) predicted all-cause and CVD-related mortality (HR 1.62 and 1.71, respectively), but not mortality from non-CVD causes. The smaller study by Haring *et al.* showed equivocal results: higher baseline total T was associated with lower mortality over 5 years, but the result was not deemed statistically significant and no associations were found at 10 years.[76] Therefore while multiple cohort studies have been reported with varying results, there are data from several large studies identifying lower endogenous levels of total or free T as independent predictors of all-cause or CVD-related deaths in middle-aged and older men.[31,71,77,78,81,85–87] Additional studies are needed to clarify associations of circulating DHT and E2 with these outcomes.

Recently, Shores *et al.* reported another observational study based on the Veterans Affairs clinical database which employed an innovative strategy to compare men who received T therapy with those who did not.[88] There were 1031 men aged 62.1 years with low T levels (<8.7 nmol l^{-1}) and no history of prostate cancer. Of these, 398 had been prescribed T treatment with a mean of 20 months duration. Men who received T therapy had lower mortality compared to 633 untreated men over 40.5 months follow-up (10.3% *vs* 20.7%, $P < 0.001$).[88] After adjusting for potential confounders, men treated with T had lower mortality risk (HR 0.61, 95% CI 0.42–0.88); which was particularly noticeable in men with prevalent diabetes (HR 0.44, 95% CI 0.23–0.84, no diabetes HR 0.72, 95% CI 0.46–1.13). This study has notable limitations which have been previously discussed.[89,90] There was no randomization, treated men were younger, and physicians could have selected healthier men for T therapy or not considered it in men who were less well. Clinical data were limited, duration of therapy was relatively short and cause-specific mortality was not reported. Nevertheless, this study does provide some reassurance as T therapy was associated with a better prognosis. The authors correctly

cautioned that the results needed to be viewed cautiously and could not be interpreted as showing beneficial effects of T treatment or as establishing a causal relationship between treatment and outcome.[88] Randomized placebo-controlled clinical trials of T therapy are still required to clarify the role of hormonal intervention in ageing men.

RANDOMISED CONTROLLED TRIALS OF TESTOSTERONE AND CARDIOVASCULAR RISK

Large placebo-controlled, RCTs of T therapy with the prespecified endpoint of CVD events in middle-aged and older men are lacking.[12] Reported clinical trials have been smaller, tending to examine surrogate endpoints with limited power for clinical outcome events.[12,17] T exhibits anti-inflammatory effects, enhances flow-mediated brachial artery reactivity, and reduces arterial stiffness.[39,91–94] Short-term T therapy had a beneficial effect on exercise-induced myocardial ischemia in middle-aged men with coronary artery disease or chronic stable angina,[95–97] and reduced angina frequency in older men with diabetes and coronary artery disease.[98] Other studies in this area have been reviewed previously.[17,99,100] Corona *et al.* performed a meta-analysis of six randomized controlled trials involving a total of 128 men given T and 129 placebo recipients.[101] Selection criteria were prevalent coronary heart disease and reporting of treadmill test outcomes. T therapy resulted in an increase in treadmill test duration and time to ST segment depression.[101] Therefore, there are interventional studies supporting a protective effect of exogenous T against myocardial ischemia in men with coronary artery disease. However, adequately powered RCTs specifically addressing this outcome are required. Such studies would be logistically challenging as large numbers of men would need to be randomized and treated for an extended duration.

In this context, the report by Basaria *et al.* is noteworthy.[102] That study, the T in Older Men with Mobility Limitations (TOM) trial randomized men aged 65 years or older with limitations in mobility and total T levels between 3.5 and 12.1 nmol l^{-1} or free T <173 pmol l^{-1} to placebo or T gel (100 mg d^{-1}) for 6 months. The participating men had a high prevalence of hypertension, obesity, diabetes, hyperlipidemia, and known CVD.[102] The study was terminated after 209 of the planned 252 men were enrolled. One hundred and twenty-nine men had completed the 6-month intervention period, and 47 had received study medication for 12 or more weeks. The average age of the men participating was 74 years and T was increased if levels of total T were <17.4 nmol l^{-1} or decreased if > 34.7 nmol l^{-1}, with mean T levels being 19.9 ± 14.0 nmol l^{-1} in the T group and 10.1 ± 5.6 nmol l^{-1} in the placebo arm. Men in the T arm reported a higher incidence of cardiovascular adverse events defined broadly to include peripheral edema, hypertension, arrhythmia, and syncope ($n = 23$ *vs* 5) and major events including acute coronary syndrome, myocardial infarction, sudden death, coronary bypass surgery, and stroke or carotid plaque ($n = 7$ *vs* 1).[102] By contrast, Srinivas-Shankar *et al.* conducted a similar study in intermediate-frail or frail men aged ≥65 years with total T <12 nmol l^{-1} or free T <250 pmol l^{-1}.[103] Men were randomized to placebo or T gel (50 mg d^{-1}) for 6 months with dose adjustments aiming for a range of 18–30 nmol l^{-1}; and from 274 men randomized, 262 completed the study. Total T levels were 18.4 ± 9.2 nmol l^{-1} in the T group and 10.7 ± 3.5 nmol l^{-1} in the placebo group. Three men in the placebo group and six in the T group reported serious adverse events with no signal for excess cardiovascular adverse events.[103] The previous studies in men with coronary heart disease[92,93,95–98,101] and the findings of Srinivas-Shankar *et al.*[103] provide a distinct contrast to the results of the TOM trial. Men randomized to T in both the Srinivas-Shankar study and the TOM trial had improved physical function.[103,104] Thus, it is conceivable that in the TOM trial T-treated men might have engaged in more strenuous activities and thereby unmasked preexisting CVD by provoking exertional symptoms. A prudent approach to T therapy in older men would be to carefully consider the benefits and risks, address risk factors, and prevalent CVD; and if therapy is indicated to employ conservative doses avoiding marked fluctuations in T levels.[105]

In the absence of randomized controlled trials with the pre-specified outcome of CVD events, existing studies of T therapy have been scrutinized for reported cardiovascular adverse events.[106–109] Four recent meta-analyses examining the occurrence of cardiovascular adverse events in men treated with T compared to those receiving placebo are shown in **Table 5**. Calof *et al.* included 19 studies published between 1966 and April 2004 of men aged ≥45 years treated for at least 90 days.[106] The rates for all CVD events including atrial fibrillation, arrhythmia, myocardial infarction, chest pain, angina, coronary procedures including bypass grafting, and vascular events including stroke were 33.2 per 1000 patient-years in the T group and 44.3 per 1000 patient-years in placebo recipients, the difference being not statistically significant. Haddad *et al.* reported studies published between 1966 and October 2004, numbering 30 trials in which cardiovascular adverse events or surrogate endpoints were reported.[107] Of six trials in which cardiovascular events were reported, 14 events (including five myocardial infarctions and one death) occurred in 161 men who received T and seven events (including two myocardial infarctions and one death) in 147 men in the control groups, the difference being not statistically significant. Fernandez-Balsells *et al.* reviewed available literature from 2003 to August 2008, identifying 51 studies with follow-up varying from 3 months to 3 years.[108] There were no significant differences in the rates of all-cause mortality, arrhythmia, coronary bypass surgery, or myocardial infarction between T and control arms. Interestingly, Xu *et al.* published a meta-analysis including 27 trials published until 31 December 2012 reporting cardiovascular-related events by study arm.[109] There were 12 studies which were published in 2009 or later, including both the Basaria and the Srinivas-Shankar trials. The OR for broadly-defined cardiovascular-related adverse events was 1.54 with a 95% CI of 1.09–2.18.[109] There were 115 events in 1733 men receiving T (6.6 per 100 men) and 65 in 1261 men receiving placebo (5.2 per 100 men). Inspection of the Forest plot shows the Basaria study to be an outlier, being the only study where the CI for cardiovascular-related events did not cross 1. The authors did not offer a sensitivity analysis excluding this study to determine whether it influenced the results of the meta-analysis as a

Table 5: Recent meta-analytic studies examining the occurrence of cardiovascular adverse events in clinical trials of testosterone therapy in men

Study author and year [ref. no.]	*Number of RCTs*	*Men on T (n)*	*Men on placebo (n)*	*Results (T vs placebo)*
Calof *et al.* 2005[106]	19[a]	651	433	Higher rate of all prostate events (OR: 1.78) and hematocrit >0.50 (OR: 3.69). No difference in cardiovascular events or mortality
Haddad *et al.* 2007[107]	30	808	834	No significant changes in blood pressure, lipids, or cardiovascular events
Fernandez-Balsells *et al.* 2010[108]	51	2716 (total)		Increased hematocrit (3.2%) and decreased HDL (–0.01 mmol l^{-1}). No significant effect on prostate, cardiovascular outcomes or mortality
Xu *et al.* 2013[109]	27[b]	1733	1261	Higher risk of cardiovascular-related events (OR: 1.54)

HDL: high density lipoprotein; OR: odds ratio; RCT: randomized clinical trial; T: testosterone. [a]Men≥45 years; duration≥90 days. [b]Trials of 12+weeks duration

whole.[109] Meta-analyses can be limited by methodological issues including difficulties in adequately accounting for heterogeneity between included studies and inconsistencies in the reporting of adverse events.[110–113] Nevertheless, the results of these studies support the concept of careful consideration of the risks and benefits of T therapy, particularly in older men where medical comorbidities are common.[114] Additional data from ongoing interventional studies of T in middle-aged and older men may help to resolve these considerations.[8] However, additional adequately powered randomized controlled trials of T with prespecified endpoints relating to cardiovascular risk or incident CVD events are needed to clarify the effects of T therapy in this setting.

CONCLUSIONS

Observational studies indicate that lower levels of endogenous T in older men are associated with the presence of carotid atherosclerosis, aortic and peripheral vascular disease, and incidence of CVD events and mortality. The role of its metabolites DHT and E2 with respect to CVD risk require clarification. Interventional studies have shown beneficial effects of exogenous T on vascular function and on exercise-induced myocardial ischemia in men with coronary artery disease. However, cardiovascular adverse events have been reported in older men with limited mobility and preexisting CVD treated with T. Further studies using accurate hormonal assays are needed to clarify the utility of T, DHT, and E2 as independent predictors of health outcomes in ageing men. Adequately powered randomized controlled clinical trials of T therapy in middle-aged and older men are required to clarify whether hormonal intervention would reduce cardiovascular risk. Men with pathologically-based hypogonadism due to pituitary or testicular disease merit consideration for T therapy. The evidence base for the use of T in older men with low levels of endogenous T is still evolving, particularly with respect to treatment-related effects on cardiovascular risk. In all men the indications for, and the risks and benefits of, T treatment should be carefully considered.

COMPETING INTERESTS

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